What is Drug Antibody Ratio and How Does It Relate to Biotherapeutics?

Meet James Song, R&D Research Scientist—Bioseparations

James has over 20 years of experience in the arena of biotechnology and has a wealth of experience in the fields of diagnostic medical devices, oncology and has recently come to Phenomenex from a biopharmaceutical company developing antibody drug conjugates (ADCs) for cancer therapeutics.

With a BS in Biology and a minor in Chemistry from the University of Houston and after decades of working with LC chromatography, James is delighted to be “on the inside” at Phenomenex developing columns that he formerly used extensively but had limited knowledge of how these products were put together.

So what better person to ask all our Drug Antibody Ratio (DAR) questions to!

1. **What exactly is DAR and how does it relate to biotherapeutics?**

DAR stands for Drug Antibody Ratio and refers to the average drug to antibody ratio for a given preparation of antibody drug conjugate (ADC). The significance of DAR in biotherapeutics is primarily as a measure of drug loading for an ADC.

For a heterogeneous population of drug loaded antibody, the distribution of drug can impact the therapeutic efficacy of an ADC and properties such as drug clearance, pharmacokinetics, and biodistribution. This impact creates a necessity for analytical scientists to determine DAR with high precision for all marketed biotherapeutics.

**Discover condensed Drug-Antibody Ratio (DAR) Tips!**

2. **There is more than one method to determine average DAR. Can you provide an overview of the techniques out there?**
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Analytical methods, ranging from as basic as UV-Vis spectroscopy through UV-HPLC and as sophisticated as LC-MS/MS, can be used to determine average DAR.

UV-Vis spectroscopy is dependent on differential absorbance maxima for the antibody and drug. For instance, the antibody absorbance maxima will invariably be 280 nm and, if the drug has an absorbance maximum at 250 nm, the relative increase of absorbance at 250 nm vs 280 nm of an ADC preparation will indicate an increased amount of drug present.

HPLC techniques such as HIC (hydrophobic interaction chromatography) and RP (reversed phase) are also used to determine DAR. These techniques work by separating different species of drug conjugated antibody and calculating the contributing proportions of drug conjugated antibody from the whole. HIC is performed under native conditions at physiological pH’s and RP is performed under reducing conditions at acidic pH’s.

Mass spectrometry methods to determine DAR include intact ADC LC-TOF, proteolytic digest of ADC LC-MS/MS assays and ion mobility MS.

The method used to determine DAR will vary with respect to the chemical properties of the cytotoxin, linker (payload) and the chemistry used to attach the payload to the antibody. Lysine conjugates because of their highly heterogenous nature are less amenable to HPLC characterization methods, therefore UV-Vis spectroscopy or MS techniques may be used to measure DAR. Cysteine conjugates or site-specific conjugates on the other hand are better suited to be analyzed by HPLC methods.

3. What is your favorite technique to assess DAR and why?

My favorite technique is RP HPLC because it is relatively broad in coverage for different types of conjugation chemistries and its limitations are more than compensated by its robust
and highly reproducible nature. That being said, you cannot solely rely upon one method but should have orthogonal methods to properly analyze a sample.

4. Do you have any tips for researchers just starting out in ADC characterization?

Many if not all of the answers to questions novices to ADC characterization might ask, have been studied and published extensively in literature.\(^1\/2\/3\) Also, take into consideration the antibody characteristics and not just the payload characteristics when deciding which techniques to measure DAR of an ADC. And lastly coming back to the question about my favorite technique, have multiple methods to measure DAR even if one is the “workhorse” that gets used the most.

5. What challenges do you predict for the determination of average DAR for future iterations of ADCs?

In one aspect the analysis of DAR will be simpler as more innovators adopt site specific technologies for conjugation to increase therapeutic efficacy and lower toxicity, pushing ADCs to greater and greater homogeneity.

But with increasing payload complexity with respect to novel cytotoxins, and linker chemistries including branched linkers as well as conjugation methods such as enzymatic linkages, the trend will move DAR determination more towards LC-MS technologies to take advantage of the high-resolution analytical capability that LC-MS provides.

References
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James Song, R&D Scientist here at Phenomenex, shares insight into drug antibody ratios or DAR and how they relate to the industry of biotherapeutics.